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Mature Cells Attracting Cells of the Complementary Mating Type in *Euplotes woodruffi* syngen 3 (Ciliophora, Hypotrichida)

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ABSTRACT—A trap for attracting ciliate cells was devised. By using the trap and cells of *Euplotes woodruffi* syngen 3, effects of gamone-like substance on attraction of cells were studied. Various types of cells such as the same mating type cells, complementary mating type cells, conjugating pairs, exconjugants and immature cells were used as bait in the trap. The cells were prepared for bait by freeze-thawing. Mature cells were attracted to the complementary mating type cells, but not to the same mating type cells and immature cells. G1 phase cells as well as late S to D phase cells attracted the complementary mating type cells. Conjugating pairs, both selfing and heterotypic pairs, and even exconjugant cells had strong attraction for mature cells. Exconjugant cells which were at 6 cell divisions after conjugation had lost their attracting ability. Immature cells, on the contrary, showed no interest in mature cells. Food organisms for bait gathered both mature and immature cells more rapidly than mature cells did. Preliminary studies showed that mature cells of *E. woodruffi* syngen 3 had no effect of attraction of mature cells of *E. woodruffi* syngen 1 and *E. aediculatus*.

INTRODUCTION

In the heterotrichous ciliate, *Blepharisma japonicum* [1, 2], the gymnostome ciliate, *Dileptus anser* [3] and the hypotrichous ciliates, *Euplotes patella* [4], *E. octocarinatus* [5], *E. raikovi* [6], and *Oxytricha bifaria* [7], it is known that mature cells excrete gamones or mating type pheromones into their culture medium, which make G1 cells of the complementary mating types prepare for and induce conjugation. In these species, conjugation occurs following recognition of the complementary mating type cells by gamones.

When mature cells excrete gamones into their surrounding water in nature, it would seem logical that the complementary mating type cells recognize the gamones and approach closely the origin of the gamones. Although many studies have been done about inducing conjugation by gamones and the chemical properties of gamones [1, 2, 8, 9, 11–14], it has only been proven in *B. japonicum* that gamones actually attract the complementary mating type cells [10].

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It has been reported in *Euplotes woodruffi* syngen 3 that mature cells not only in G1 phase but also in S and D phases induce selfing [15]. The implication that even S and D phase cells excrete gamones, which is very different from the results in the other *Euplotes* species, indicate that gamones might have an important effect in addition to making competent cells undergo conjugation. In other words, gamones might have another effect by increasing the cell density and increasing the chance for mating by inducing complementary mating type cells to approach each other closely.

To test this possibility, a method for attracting cells by gamones was devised. By using this method and many kinds of cells which were in immaturity or maturity, in G1 phase or late S to D phase, in conjugation, and exconjugants, attraction of cells was examined.

MATERIALS AND METHODS

Stocks and culture conditions

The stocks used in this experiment were three complementary mating type stocks, SJ-45, SJ-24 and EZL-7, of *Euplotes woodruffi* syngen 3. Of

these stocks, stock EZL-7 frequently underwent selfing in cultures. All stocks were cultured and kept at 20–21°C in a wheat infusion (40 wheat grains per 1 of distilled water, boiled for 10 min), and were fed with the colorless flagellate *Chilomonas paramecium* which was cultivated in the same medium. In addition to these stocks of *E. woodruffi* syngen 3, two stocks of *E. woodruffi* syngen 1 and two stocks of *E. aediculatus* (all in maturity) were used.

Methods for attracting cells by various types of cells as baits

For testing the attraction of cells, an apparatus was devised for this purpose, and is shown in Figures 1 and 2. In a glass Petri dish which is 9 cm in diameter, 40 ml of 0.05% Van't Hoff artificial sea water was contained as an experimental solution. 400 living cells which were in the mature or immature periods were added to the solution after washing two times by the same solution. To trap the living cells, hollow glass tubes of 3 cm in length were used (Fig. 2). In the hollow glass tubes, absorbent cotton to which had been attached various types of cells as bait was set. The baits, which were prepared by freeze-thawing, were cells of the same mating type or complementary mating types, selfing pairs, heterotypic conjugating pairs, exconjugants and immature cells, because it is difficult to keep living cells in glass tubes. Thirty G1 phase or 15 S to D phase cells in 20 μl of an experimental solution were frozen and thawed, and used as the freeze-thawed cells (lysed cells) which were absorbed to a small amount of absorbent cotton. Then the cotton was inserted into a glass tube by using a teasing needle. The glass tube was filled with an experimental solution, and settled in a Petri dish. It has already been reported that the lysed cells as well as cell-free fluid and living cells have the same effects in inducing conjugation [15]. For obtaining selfing pairs, Kosaka's method for inducing selfing was used [15].

FIG. 1. An apparatus for attracting living cells. Arrows show marks on the fishing line. By the marks, the kinds of bait are easily distinguished. Usually, two to four glass tubes are put in the Petri dish. Here three tubes which are settled at regular intervals are seen.

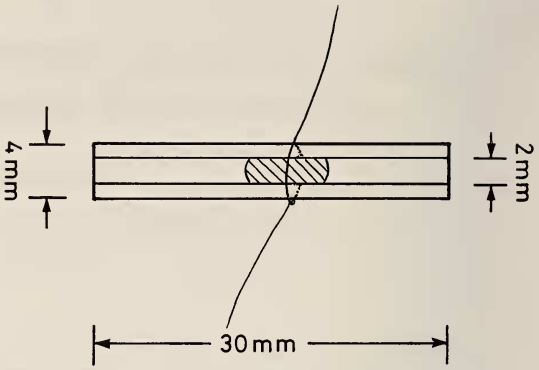

FIG. 2. A glass tube for trapping living cells. Absorbent cotton to which were absorbed various kinds of cells is set in the center of the tube for bait. Cotton is also needed in the control experiments, because cells gathered in the tube are easily pushed off the tube by a current without cotton in the tube. The center of the tube is tied with a fishing line. The line is very important to fix the glass tube on the bottom of the Petri dish. Without the line, the glass tube rolls on the Petri dish, and frequently crushes euplotes creeping on the bottom of the dish.

To avoid the effect of light, the Petri dishes were put into a light-tight metal box. Then the box was put in the incubator at 20–21°C.

To know when the effect of bait should be measured, the attraction of cells by bait with time was examined. The results are shown in Figure 3. The results show that the maximum peak of the

TABLE 1. Effect of baits prepared with various number of freeze-thawed cells on the attraction of living cells

Stock used	No. of cells for bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	5	31 (2)	32 (0)
	10	39 (2)	54 (2)
	20	37 (2)	73 (2)
	30	40 (1)	85 (0)
SJ-24	5	26 (5)	5 (0)
	10	16 (3)	36 (3)
	20	39 (8)	55 (5)
	30	41 (6)	47 (4)

Cells from stock EZL-7 were used as bait. Attracted cells from control experiments in which only absorbent cotton was used as bait are shown in parentheses.

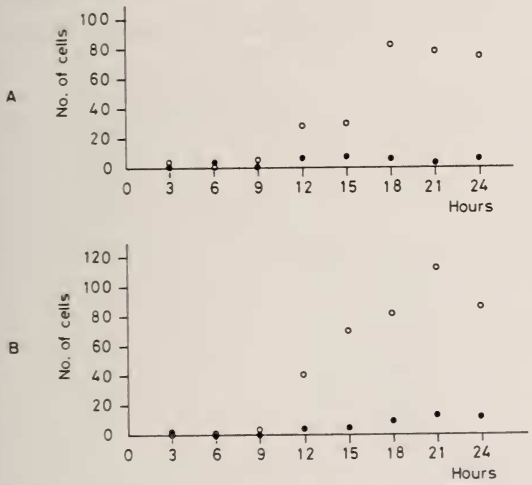

FIG. 3. Attraction of living cells into a trap by bait with time. A: Living cells were from stock SJ-45. The bait was prepared from the cells of stock EZL-7 (○). Only absorbent cotton was used in the control experiment (●). B: Living cells were from stock SJ-24. The bait (○) and the control (●) are the same as Fig. 3A.

effect of bait was seen between 18 and 24 hr. Following these results, cells gathered in the glass tube were counted 20 to 21 hr after the start of the experiment.

The effect of the number of bait cells on the attraction of living cells were studied, and are shown in Table 1. The results show that from 5 to 30 cells had an attracting ability, and that 30 cells were the most effective among them. Therefore 30 cells were used in each bait for attracting cells. However, in the case of conjugating pairs, 15 pairs were used instead.

RESULTS

Attraction of mature cells by complementary mating type cells

Living cells from three stocks, SJ-45, SJ-24 and EZL-7, were used to test the effect of one mating type cells on the complementary mating type cells. Three different baits were used: one of the same mating type, one of the complementary mating type and one control. The results from two experiments are shown in Table 2. It is clear that living cells from each of the three stocks were attracted only to the bait made from the complementary mating type stock, and not attracted to the bait made from the same mating type stock nor to the control. Table 3 shows the results when two different mating type stocks were used simultaneously in the same dish as the baits. The results show that each of two different mating type stocks attracted the complementary mating type cells. It also seems that each mating type cells had a preference for one of the two different mating types: stock SJ-45 much preferred the bait from stock EZL-7 to that from stock SJ-24; stock SJ-24 preferred the bait from stock EZL-7 to that from

TABLE 2. Effect of baits on the attraction of living cells

Stock used	Stock source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	EZL-7	131	106
	SJ-45	3	2
	Control	4	6
SJ-24	SJ-45	46	45
	SJ-24	2	0
	Control	5	5
EZL-7	SJ-45	56	54
	EZL-7	5	3
	Control	2	2

Only absorbent cotton was used in the control experiments.

TABLE 3. Effect of baits on the attraction of living cells

Stock used	Stock source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	SJ-24	12	7
	EZL-7	118	53
	SJ-45	2	1
	Control	1	3
SJ-24	SJ-45	20	16
	EZL-7	69	124
	SJ-24	4	0
	Control	7	8
EZL-7	SJ-24	68	32
	SJ-45	48	51
	EZL-7	6	6
	Control	1	2

Only absorbent cotton was used in the control experiments.

TABLE 4. Effect of G1-phase cells or late S-phase and D-phase cells on the attraction of living cells

Stock used	Stock source of bait	Stage of cell cycle	No. of cells attracted	
			Experiment 1	Experiment 2
SJ-24	SJ-45	G1	183 (9)	158 (5)
		late S to D	77 (8)	103 (2)
SJ-45	EZL-7	G1	163 (0)	172 (0)
		late S to D	38 (6)	39 (4)

Control experiments are shown in parentheses. Fifteen late S-phase to D-phase cells were used as bait, rather than the 30 used for G1-phase cells.

stock SJ-45. However, EZL-7 cells gathered to the bait from stock SJ-24 as well as SJ-45.

The effect of cell cycle phases on the attraction of cells was studied. Two stocks were used in these experiments. When cells from stock EZL-7 were used as living cells, the cells very often underwent selfing at a high rate when induced by the baits which were made from complementary mating types. So living cells of this stock were omitted in the subsequent experiments. In these experiments, the number of cells used as bait were 30 in G1 phase, while 15 in late S to D. It is considered that one late S to D phase cell is approximately equivalent to two G1 cells, because the former is just the predividing cell. The results are shown in Table 4. The results show that both the G1 phase cells and the late S to D phase cells from the complementary mating types have an ability to attract cells.

Attraction of mature cells by conjugating pairs and exconjugants

Whether conjugating pairs and exconjugants still kept an ability for attraction of mature cells were studied. The results of the attraction of cells by conjugating pairs are shown in Table 5. The results show that both selfing pairs and heterotypic pairs kept their ability for attraction.

The attraction of cells by exconjugants prior to any fissions is shown in Table 6. The results show that the exconjugants from selfing and heterotypic pairs still kept their ability for attraction.

Studies were also done to determine whether cells in early immature period still kept an ability to gather cells. As 30 cells were needed as one unit of bait, exconjugant cells were used at about 6 cell divisions after conjugation. The results are shown in Table 7. It is seen that the cells after undergoing

TABLE 5. Effect of selfing and conjugating pairs on the attraction of living cells

Stock used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	SJ-24 (Selfing pairs)	102 (4)	115 (3)
SJ-45	EZL-7 (Selfing pairs)	105 (7)	187 (4)
SJ-45	SJ-24×EZL-7 (Conjugating pairs)	214 (6)	197 (14)
SJ-24	SJ-45×EZL-7 (Conjugating pairs)	188 (7)	192 (7)

15 pairs were used for bait at 1 to 2 hr after pair formation. Control experiments are shown in parentheses.

TABLE 6. Effect of exconjugants on the attraction of living cells

Stock used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	EZL-7 (Selfing)	103 (4)	57 (3)
	SJ-24×EZL-7 (Conjugation)	93 (4)	80 (11)
SJ-24	EZL-7 (Selfing)	111 (2)	133 (6)
	SJ-45×EZL-7 (Conjugation)	74 (3)	81 (8)

30 exconjugants were used for bait at about 20 hr after pair formation. One macronuclear anlage was seen in each exconjugant. Control experiments are shown in parentheses.

6 cell divisions had already lost an attracting ability.

In Table 8 are shown the results when immature cells as well as mature cells were used as bait at the same time. The results show that the complementary mating type cells were endowed with an attracting ability, but immature cells had no effect of attraction, when both complementary

TABLE 7. Effect of immature cells on the attraction of living cells

Stock used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	SJ-45×EZL-7	12 (10)	11 (3)
SJ-24	SJ-45×SJ-24	2 (8)	6 (7)

Immature cells were at about 6 cell division age. Control experiments are shown in parentheses.

TABLE 8. Effect of immature cells on the attraction of living cells

Stock used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	Immature cells of G1 phase[1)]	9	13
	EZL-7	157	205
	Control	11	6
SJ-45	Immature cells of G1 phase[1)]	15	18
	SJ-45	18	14
	Control	8	22
SJ-45	Immature cells of S to D phase[2)]	6	2
	EZL-7	56	51
	Control	1	3
SJ-45	Immature cells of S to D phase[2)]	4	0
	SJ-45	11	2
	Control	9	3

[1)] Immature cells were at 6 cell division age and originated from an exconjugant clone from a cross between stock SJ-45 and stock EZL-7.
[2)] 15 late S-phase to D-phase cells were used for bait, and originated from the same clones as[1)].

TABLE 9. Effect of mature cells on the attraction of immature cells

Immature clone used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
Clone 5	Immature cells	1	1
	SJ-45	1	5
	Control	3	3
Clone 5	Immature cells	5	4
	EZL-7	10	5
	Control	9	0

Immature clones originated from a cross between stock SJ-45 and stock EZL-7, and were within 15 fission age. Immature cells were the same as those in TABLE 8.

mating type cells and immature cells were in existence in the same place. On the other hand, when there were both immature cells and the same mating type cells, both types of cells failed to attract any cells. These results also indicate that the immature cells had already lost their attracting ability toward mature cells by the 6th cell division.

Late S to D phase cells in the immature period were used as bait instead of G1 phase cells. The results are shown in Table 8. From the results, it is seen that the immature cells of late S to D phase had no attracting effect on mature cells. These results and the results mentioned earlier show that cells from the immature period lost their attracting ability throughout all stages of the cell cycle.

The reverse situation, whether mature cells attract immature cells was studied. The results in Table 9 show that the immature cells were not attracted to the mature cells.

Attraction of cells by food organisms

Attraction of immature and mature cells by food organisms was studied. At first, the relation between time and the number of cells gathered toward food organisms was examined, and shown in Figure 4. In these experiments, four traps were used: one containing living food organisms, one with complementary mating type cells, one with the same mating type cells and one control. From the results, it is seen that the food organisms gathered cells far faster than the other baits. The attraction of the complementary mating type cells seemed to usually occur 12 hours after treatment (Fig. 3). The results of the attraction of cells by food organisms, complementary mating type cells and the same mating type cells are shown in Table 10. From the results, it is seen that food organisms

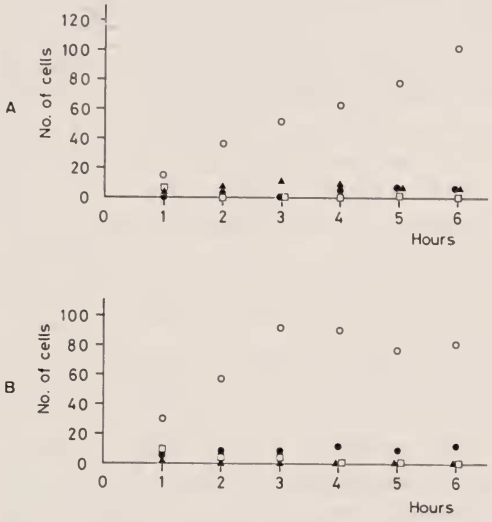

FIG. 4. Attraction of living cells into a trap by various baits with time. A: Experiment 1. B: Experiment 2. Cells of stock SJ-45 were used as living ones in the two experiments. The baits used were as follows: 20 μl of concentrated *Chilomonas paramecium* as a food organism (○); cells of stock EZL-7 (●); cells of stock SJ-45 (▲); absorbent cotton only (□).

TABLE 10. Effect of food organisms on the attraction of living cells

Stock or clone used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
SJ-45	EZL-7	77	73
	SJ-45	0	4
	Food	116	151
	Control	4	4
SJ-24	SJ-45	36	35
	SJ-24	11	2
	Food	74	81
	Control	10	5
Immature clone	Food	102	94
	Control	7	2

Living *Chilomonas* which were permeated into absorbent cotton were used as food organisms. Immature clone was the same as in TABLE 9.

gathered more cells than the complementary mating type cells did when food organisms and the complementary mating type cells coexisted. Moreover, it is important to notice the fact that immature cells which could not be attracted by mature cells gathered to food organisms.

Effect of baits on attracting cells in a larger container

Does this type of trap used in these experiments really reflect the effect in nature? Preliminary study was done to know the effect of trap by using a larger container. Methods for the experiment are shown in Figure 5 where a container with 2 l of an experimental solution and eight traps are seen.

To approach the situation of cell density in nature, the density was lowered from 10 cells/ml to 1 cell/ml. To avoid the effect of light, a container made of stainless steel which shut off light completely was used. The results are shown in Table 11. From the results, attraction of cells by the other mating type cells occurred in such a larger container and lower cell density. The results also may show the possibility of applying this type of trap to gather cells in nature.

Effect of the cells of Euplotes woodruffi syngen 3 on the cells of closely related species

Do mature cells of *E. woodruffi* syngen 3 gather cells of other *Euplotes* species? To answer this question, a study was made. Living cells originating from two complementary mating types of *E. woodruffi* syngen 1 and those of *E. aediculatus* were used. Two different baits made from mature cells of two stocks of *E. woodruffi* syngen 3 were used. The results are shown in Table 12. From the results, it is clear that cells of *E. woodruffi* syngen 3 had no attracting effect on the cells of *E. woodruffi* syngen 1 and *E. aediculatus*. It seems that attraction of cells by mature complementary type cells is species specific.

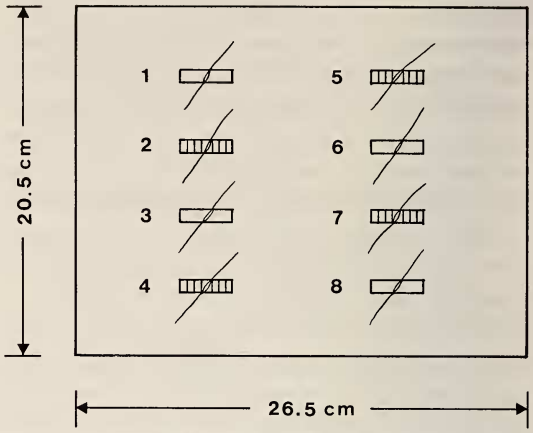

FIG. 5. Arrangement of glass tubes for traps in a larger container. Cells of stock SJ-24 were used as living ones. Numbers 1, 3, 6 and 8 of the glass tubes contained the bait prepared from cells of stock SJ-45, while the others had only absorbent cotton in the glass tubes.

DISCUSSION

The purpose of these experiments was to investigate whether mature cells of one mating type could attract mature cells of complementary mating types. For understanding how conjugation actual-

TABLE 11. Effect of bait on the attraction of living cells in a larger container

Stock used	Source of bait		No. of cells attracted	
			Experiment 1	Experiment 2
SJ-24	SJ-45	No. 1	12	20
		No. 3	12	15
		No. 6	7	14
		No. 8	10	6
	Control	No. 2	0	1
		No. 4	0	1
		No. 5	0	1
		No. 7	1	0

TABLE 12. Effect of the cells of *Euplotes woodruffi* syngen 3 on closely related species

Stock used	Source of bait	No. of cells attracted	
		Experiment 1	Experiment 2
E. woodruffi syngen 1	*E. woodruffi* syngen 3		
MY-1	SJ-45	2	11
M-25		4	7
Control		5	4
E. woodruffi syngen 1	*E. woodruffi* syngen 3		
MY-1	SJ-24	13	2
M-25		12	5
Control		18	7
E. aediculatus Sae-5	*E. woodruffi* syngen 3	2	3
EZae-1	SJ-45	1	9
Control		3	8
E. aediculatus Sae-5	*E. woodruffi* syngen 3	4	2
EZae-1	SJ-24	6	3
Control		4	5

Only absorbent cotton was used in the control experiments.

ly occurs in nature, it is necessary to determine how mature cells approach and encounter cells of complementary mating types. In *Blepharisma japonicum*, gamone 2 attracts complementary mating type cells [10]. However, no attempt to study this question has been made in other ciliate species. The results from the present experiments have made it clear that mature cells can attract cells of the complementary mating types: mature cells of complementary mating types, releasing gamones into their surroundings, affect each other and increase their cell density to improve their chances for mating. This finding that mature cells attract complementary mating type cells is the first one in the hypotrichous ciliates.

Lysed cells instead of cell-free fluids (= gamones) and living cells were used in these experiments, because lysed cells were the easiest to handle among the three. It has already been reported that lysed cells as well as cell-free fluids and living cells have an ability to induce mating [15]. This indicates that lysed cells have an ability to induce mating as gamones do. Moreover, lysed cells of one mating type attracted only the complementary mating type cells, but not the same mating type cells. Lysed cells from the immature period have no effect on attracting mature cells. For these reasons, the substances released from lysed cells of the mature period, which induce mating in the complementary mating type cells and attract them, might be considered to be gamones.

Attraction of mature cells were made by not only lysed G1 phase cells but also lysed late S to D phase cells. However, mature cells were not attracted by lysed cells from the immature period. These results seem to be closely related to the finding that lysed cells of G1, S, and D phases in maturity induce mating in complementary mating type cells [15]. Because species in nature seem to live in a low population density, it is reasonable to consider that mature cells are releasing gamones into their surroundings throughout the entire cell cycle to attract mature cells toward one another and aid them in carrying out conjugation. Using the gamones, dispersion of mature cells also might be prevented.

Mating pairs and exconjugants still have an attracting ability. Although pairs and exconjugants could not undergo further mating for structural reasons, that they maintain their attractiveness seems to be important. As immature cells lost an attracting ability within 6 cell divisions after

conjugation, the production of gamones must be stopped at the early stage of immaturity in the life cycle. From the results that pairs and exconjugants but no immature cells maintained the attracting ability, it could be presumed that both pairs and exconjugants still release gamones into their surroundings.

Mature cells attracted neither cells of the same mating type nor cells in the immature period. These results show that cells of the same mating type seem apparently to be similar to those of the immature period. However, as cells of the same mating type could attract cells of the complementary mating types, there clearly are differences between the cells of maturity and those of immaturity. This may be caused by an absence of receptor(s) for the gamone(s) in cells of the immature period. It is known in *Euplotes octocarinatus* that cells express no gamones and gamone-specific receptors during the immature period [16]. A situation similar to that in *E. octocarinatus* may be present in *E. woodruffi* syngen 3 as well.

That gamones induce complementary mating type cells to prepare for mating is true [1–7]. However, in the earlier laboratory works, experiments were done in a situation of a higher cell density (1,000 to 10,000 cells/ml in *Blepharisma japonicum, Oxytricha bifaria, Euplotes octocarinatus* and *E. raikovi*) [2, 5–7, 9]. Also gamones were obtained from cultures of a higher cell density. On the other hand, from my experience, species similar to these in size have never been observed at such a high cell density in nature. For example, in the *Euplotes woodruffi* complex, a cell density of each syngen was under 1 cell/ml at the densest. Also, natural population of any one species by no

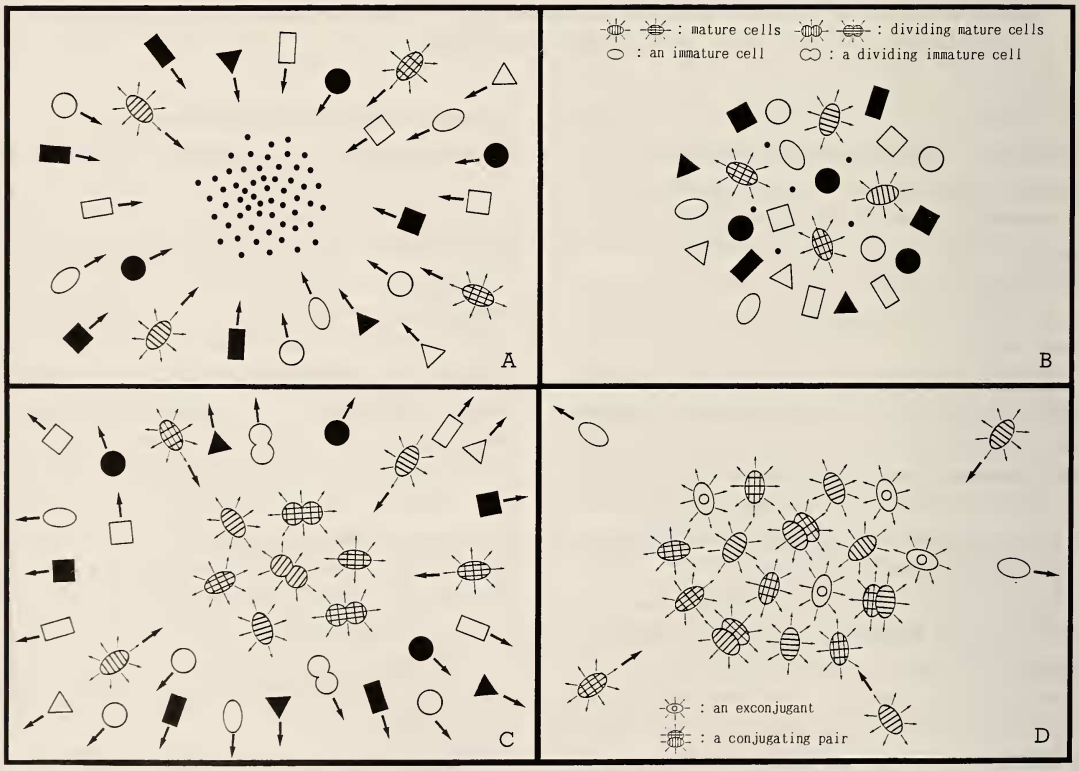

FIG. 6. Possible processes to conjugation in nature. One species is figured as the nucleus of the ciliate population here. A: attraction of many ciliate species by food organisms; B: a ciliate population consisting of many species; C: dispersion of many species. Many other species and immature cells of the same species are leaving the area, while mature cells of the same species still remain and even are gathering to increase their density; D: formation of conjugating pairs. Small arrows around cells indicate excretion of gamone(s). The other marks (■, □, ▲, △, ○, ●, ▭, ▬) show the other species.

means consisted only of mature cells. The total of adolescent cells and mature cells in general never exceed 50% of cells in any one population [17, unpublished data]. Furthermore, many other species are living together in the same place. Judging from these observations, the situation used in the earlier laboratory works (two different mating types being mixed under very high cell density or very high concentration of gamones being mixed with a high density of cells) actually never occurred in nature.

However, occurrence of mating in nature is also true. Sexual reproduction is one of the most important processes for rejuvenation: species with an ability for sexual reproduction, either conjugation or autogamy, age when undergoing only binary cell divisions, and finally die [18–25]. Then, how do mature cells find their mates and complete sexual reproduction? A possible process, which is shown in Figure 6, is presented based upon the results that mature cells attracted the complementary mating type cells of the same species. First cells of several species which are in various life cycle stages are attracted by food organisms (Fig. 6A). This hypothesis is well-grounded: Food organisms attract cells, both immature and mature, of all mating types. Thereafter, many species gathered around food organisms (Fig. 6B). As a result of gathering, a population consisting of many species in immaturity or maturity is made and is distributed in a patchy cluster. It is known that ciliates are distributed and live in patchy clusters [26–28]. Cell density has to become denser initially. Cells approaching each other more closely make possible the recognition of gamones of the complementary mating types by mature cells. Cells that have finished eating food organisms begin to carry out cell divisions, further increasing cell density (Fig. 6C). As both G1 phase and dividing mature cells excrete gamones, mature cells approach the cells excreting different gamones. In *Blepharisma japonicum*, it has been reported that type I cells respond to extremely low concentration of gamone 2 [10]. Immature cells of the same species as well as the other species start dispersing to find new food organisms. It is expected that the approach of more mature cells and maintaining them in same area continues to increase the concentration of gamones in the surroundings. This situation also may stimulate complementary mature cells which are scattered around food organisms to respond and make them add to the mature population. Finally, conjugation begins to occur between complementary mating type cells of the same species (Fig. 6D). It seems appropriate to think that conjugating pairs start to be made when food organisms are exhausted by ciliate species in a patchy cluster, because conjugation generally occurs between mature cells when a culture condition is rapidly changed from an abundant food condition to a food-poor condition. As conjugating pairs and probably exconjugants also were releasing gamones into surroundings, a concentration of gamones around a cell population will become stronger. This would draw more mature cells to the cell population. Immature cells after conjugation, which had already lost an attracting ability at the 6th postzygotic cell division at most, are no longer kept there by gamones, and then leave the place to seek food organisms in the early stage of life cycle.

Lysed cells of one mating type have attracted selectively complementary mating type cells of the same species. This is the first attempt in ciliates to gather mature cells by lysed cells of complementary mating types. When this method is applied in nature in future, it is anticipated that mature cells will be able to be gathered effectively by traps.

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